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Protective effect of resveratrol against hepatic damage induced by heat stress in a rat model is associated with the regulation of oxidative stress and inflammation

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ABSTRACT

Heat stress jeopardizes humans and animals health, and results in enormous economic loss in public health care and livestock production. The aim of this study was to investigate the effects of resveratrol on hepatic oxidative stress and inflammation in heat-stressed rats. Male Sprague-Dawley rats were orally fed with 100 mg resveratrol/kg body weight/day prior to heat stress (40 ∘C per day for 1.5 h) exposure for 3 consecutive days. Serum and liver samples were collected for the analysis of hepatic injury, redox status and immune response. The results showed that the heat-stress-induced increased aspartate aminotransferase activities in the serum, aberrant hepatic histology, excessive hepatic malondialdehyde and tumor necrosis factor alpha concentrations, and up-regulation of heat shock protein 70, superoxide dismutase 1, glutathione peroxidase 1, toll-like receptor 4 and interleukin 10 mRNA expression in the liver were mitigated by oral resveratrol treatment. Collectively, the beneficial effects of resveratrol on hepatic damage induced by heat stress were associated with the regulation of oxidative stress and inflammation.

1. Introduction

Heat stress (HS) jeopardizes humans and animals health, and results in enormous economic loss in public health care and livestock production. Moreover, the unfavorable influence of HS will be progressively deteriorating with the development of global warming (Bouchama and Knochel, 2015). HS leads to oxidative stress (OS) demonstrated by the excessive reactive oxygen species (ROS) production and the impaired antioxidant capacity (Song et al., 2018). OS can induce the damage of biomacromolecules such as DNA, proteins and lipids, and further results in cell dysfunction and tissues injury (Cheng et al., 2017b). In addition, HS also causes the disruption of anti- and pro-inflammatory cytokines balance (Song et al., 2017). If inflammation occurs, particularly for livestock, the nutrients will be spent in maintaining the abnormal immune response rather than in growing, ultimately, lowering feed efficiency (Li et al., 2015). The local and systemic OS and inflammation induced by HS have been reported in a vast range of humans (Bouchama et al., 1991; Leon, 2007), livestock (e.g., broilers (Song et al., 2017, 2018), pigs (Pearce et al., 2013; Montilla et al., 2014)) and rodents (Hall et al., 2001; Kim et al., 2012, 2015) models. Particularly in the liver (a primary metabolic and detoxified organ), the overproduction of free radicals and pro-inflammatory cytokines, and the impairment of enzyme and non-enzyme antioxidants levels were also observed in the aforementioned HS models, leading to hepatic disorder. Also, it has to be mentioned here that there is a vicious circle between OS and inflammation. Thus, the maintenance and enhancement of liver function by inhibiting OS and/or inflammation in the organism are important for the prevention and/or treatment of HS.

Resveratrol (RSV, trans-3, 4′, 5 trihydroxystilbene), a polyphenol from plants, has been shown to prevent and/or treat various liver diseases partly induced by free radicals and inflammatory cytokines in vivo studies, including drug-induced hepatotoxicity (Tunali-Akbay et al., 2010), alcoholic cirrhosis (Bujanda et al., 2006) and high-fat-diet-induced liver damage (Bujanda et al., 2008). RSV is gradually regarded as a considerable promising therapeutic agent in treating the aforementioned liver ailments due to its potent antioxidant and anti-inflammatory properties (Bishayee et al., 2010). However, it is unknown whether RSV can alleviate the HS-mediated OS and inflammation in the liver of rats. Therefore, the effects of RSV on hepatic OS and inflammation in heat-stressed rats were investigated in the present study. Of course, understanding the role of RSV in HS-induced hepatic damage is beneficial for designing new therapeutic approaches in the future.

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2. Methods and materials

2.1. Animals treatment and experimental design

All procedures involving animals in the present study were performed following the Institutional Animal Care and Use Committee of Nanjing Agricultural University (Nanjing, China). A total of 24 Sprague-Dawley rats (8 weeks old, male) with a mean initial weight of 200 ± 20 g were purchased from Qinglongshan Animal Breeding Farm (Nanjing, China). All rats had free access to tap water and standard chow diet, and were maintained under the same condition with a regulated temperature (22 ± 2 °C), humidity (50 ± 10%), and light/dark cycle (light on 07:00–19:00). After 1 weeks of acclimation, rats were divided into 3 groups (n = 8) following the body weight: (1) the control (CON) group were orally fed 0.5% carboxymethylcellulose sodium (CMC-Na; Sinopharm Chemical Reagent Co., Ltd., Shanghai, China; diluted in 0.86% saline) under normal condition; (2) the HS group were subjected to 40 ∘C for 1.5 h between 11:30 and 13:00 daily for 3 consecutive days with receiving 0.5% CMC-Na by oral administration; and (3) the HS-RSV group were orally fed 100 mg RSV/kg body weight/day (purity 99%; TCI Co., Ltd., Tokyo, Japan; diluted in 0.5% CMC-Na) under HS environment. Two hours before HS treatment, CMC-Na or RSV were provided for 3 consecutive days. The RSV dose was selected as previously published study (Cheng et al., 2018).

2.2. Sample collection

After heat treatment on the 3rd day, all rats each group were sacrificed quickly and blood samples were collected through eyeballs and centrifuged (2000 g, 4 °C, 15 min) to harvest the serum. The serum was stored at −80 °C until analysis. Liver tissues were excised immediately, rinsed in ice-cold physiological saline. A fraction of liver samples from the left lobe were collected and fixed in 4% paraformaldehyde, while the remaining parts were snap-frozen in liquid nitrogen and stored at −80 °C for further analysis.

2.3. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities

The activities of ALT and AST in the serum were measured by commercial kits in accordance with the manufacturer's guidelines (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.4. Hepatic histology

After fixed, liver sample was embedded in paraffin wax, and sections in 5 μm was stained with hematoxylin and eosin (H&E) according to a standard protocol. And then, images were collected using an optical binocular microscope (Olympus BX5; Olympus Optical Co. Ltd, Tokyo, Japan) equipped with a digital camera (Nikon H550L; Nikon, Tokyo, Japan).

2.5. Hepatic redox status

In order to evaluate hepatic redox status, the antioxidants levels and lipid peroxidation were measured. The commercial kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) were employed to analyze the hepatic total superoxide dismutase (T-SOD) and glutathione peroxidase (GPX) activities, and the levels of malondialdehyde (MDA) and total antioxidant capacity (T-AOC), respectively. All results were normalized to total protein concentration in each sample for inter-sample comparison. The hepatic protein concentration was detected according to the previous method (Bradford, 1976).

2.6. Hepatic tumor necrosis factor alpha (TNF-α) concentration

Hepatic TNF-α concentration was analyzed by the enzyme-linked immunosorbent assay (ELISA; Beijing 4A Biotech Co., Ltd, Beijing, China) according to the instructions of manufacturer. The sensitivity was 15 pg/mL, and the intra-and inter-assay coefficients of variation were less than 10%. All results were normalized to total protein concentration in each sample for inter-sample comparison. The hepatic protein concentration was detected according to the previous method (Bradford, 1976).

2.7. Hepatic gene expression

The mRNA expression in liver was detected according to the method previously described (Cheng et al., 2016). In brief, total RNA isolated from the liver using TRIzol Reagent (TaKaRa, Dalian, China) according to the protocols of manufacturer. The RNA integrity was checked on 1% agarose gel with ethidium bromide staining. The RNA concentration and purity were determined from OD260/280 readings (ratio > 1.8) using a spectrophotometer (NanoDrop, 2000c; Thermo Scientific, USA). After then, total RNA (1 μg) was reverse-transcribed into cDNA using the PrimeScriptTM RT Reagent Kit (TaKaRa, Dalian, China) according to the guidelines of manufacturer.

The primer of C-C motif chemokine ligand 2 (Ccl2), GPX1, heat shock protein 70 (HSP70), interferon γ (IFN-γ), interleukin 6 (IL6), IL10, nuclear factor, erythroid 2-like 2 (Nrf2), SOD1, toll-like receptor 4 (TLR4), TNF-α, Kelch-like ECH-associated protein 1 (Keap1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and beta actin (β-actin) are given in Table 1. The qRT-PCR was performed with a SYBR Green qPCR Kit (Vazyme, Nanjing, China) on an Applied Biosystems 7500 Real-Time PCR System (Life Technologies). The PCR reaction mixture contained 2 μL of cDNA, 0.4 μL of forward primer, 0.4 μL of reverse primer, 10 μL of SYBR Premix Ex Taqe (Vazyme, Nanjing,

Table 1
Primer sequences used for qRT-PCR.

Gene	Gene bank ID	Primer sequence, sense/antisense	Length (bp)
HSP70	NM_153629.1	TCAGAGCTGCTATGTCGCTG GCAGCGGTCGCTATACTCAT	73
Ccl2	NM_031530.1	CAGGTCTCTGTCACGCTTCT GGCATTAACTGCATCTGGCTG	87
TNF-α	NM_012675.3	AACACACGAGACGCTGAAGT TCCAGTGAGTTCCGAAAGCC	93
IFN-γ	NM_138880.2	ATCCATGAGTGCTACACGCC TCGTGTTACCGTCCTTTTGC	197
IL6	NM_012589.2	ACAAGTCCGGAGAGGAGACT TTCTGACAGTGCATCATCGC	172
IL10	NM_012854.2	TGCGACGCTGTCATCGATTT GTAGATGCCGGGTGGTTCAA	186
TLR4	NM_019178.1	TCCACAAGAGCCGGAAAGTT TGAAGATGATGCCAGAGCGG	126
SOD1	NM_017050.1	GCATGGGTTCCATGTCCATC CAGGTCTCCAACATGCCTCTC	127
GPX1	NM_030826.4	GCTCACCCGCTCTTTACCTT TGGAACACCGTCTGGACCTA	162
Nrf2	NM_031789.2	TTTGTAGATGACCATGAGTCGC TGTCCTGCTGTATGCTGCTT	142
Keap1	NM_057152.2	TGTGCTGCATGTGATGAACG AAGAACTCCTCCTCCCCGAA	198
β-actin	NM_031144.3	GCAGGAGTACGATGAGTCCG ACGCAGCTCAGTAACAGTCC	74
GAPDH	NM_017008.4	AGTGCCAGCCTCGTCTCATA GGTAACCAGGCGTCCGATAC	77

Ccl2, C-C motif chemokine ligand 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GPX1, glutathione peroxidase 1; HSP70, heat shock protein 70; IFN-γ, interferon γ; IL6, interleukin 6; IL10, interleukin 10; Nrf2, nuclear factor, erythroid 2-like 2; SOD1, superoxide dismutase 1; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor alpha; Keap1, Kelch-like ECH-associated protein 1; β-actin, beta actin.

China), 0.4 μL of ROX Reference Dye (Vazyme, Nanjing, China), and 6.8 μL of double-distilled water. The PCR consisted of a pre-run at 95 °C for 30 s and forty cycles of denaturation at 95 °C for 5 s, followed by a 60 °C annealing step for 30 s. The conditions of the melting curve analysis were as follows: one cycle of denaturation at 95 °C for 10 s, followed by an increase in temperature from 65 to 95 °C at a rate of 0.5 °C/s. Each sample was run in duplicate and melt curve analysis was performed to validate the specificity of the PCR-amplified product. We found that β-Actin mRNA expression was more stable than GADPH among hepatic segments and treatments by analyzing gene stability as previously described (Vandesompele et al., 2002). After normalization against the housekeeping gene β-actin, the relative levels of mRNA expression of target gene were calculated via the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The values of CON group were used as a calibrator.

2.8. Statistical analysis

Data were analyzed by using SPSS 17.0 statistical software. Individual rat was used as the experimental unit for all statistic procedures. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Data are expressed as means and standard error. Values of $p < 0.05$ and $0.05 < p < 0.1$ were considered statistically significant and a trend, respectively.

3. Results

3.1. Hepatic HSP70 mRNA expression

In Fig. 1, heat exposure increased ($p < 0.05$) the HSP70 mRNA expression in the liver of the HS group when compared with the CON group. RSV treatment reduced ($p < 0.05$) the hepatic HSP70 mRNA expression in the HS-RSV group when compared with the HS group.

3.2. Serum AST and ALT levels

Compared with the CON group, the HS group had higher AST and ALT levels in the serum ($p < 0.05$, Fig. 2A and B). In contrast, the serum AST ($p < 0.05$) and ALT ($p = 0.054$) levels were lower in the HS-RSV group than that in the HS group.

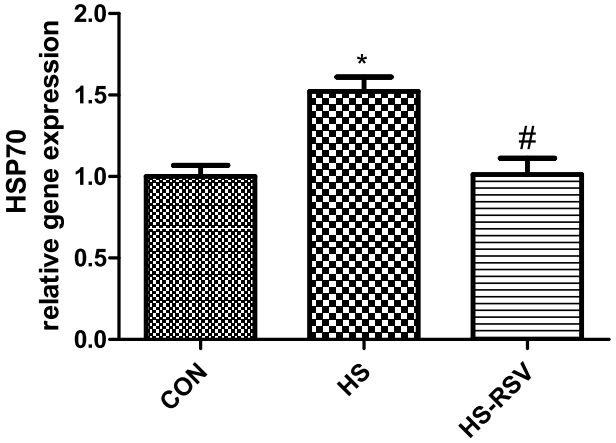

Fig. 1. Resveratrol reduces the heat-stress-induced hepatic heat shock protein 70 (HSP70) gene overexpression in rats. CON, rats were orally fed with 0.5% carboxymethylcellulose sodium under normal condition; HS, rats were orally fed with 0.5% carboxymethylcellulose sodium and challenged with heat treatment; HS-RSV, rats were orally fed with 100 mg resveratrol/kg body weight/day and challenged with heat treatment. The column and its bar represented the means value and standard error, n = 8, respectively. Significant difference is depicted as $^{*}p < 0.05$ when compared with the CON group, $^{\#}p < 0.05$ when compared with the HS group.

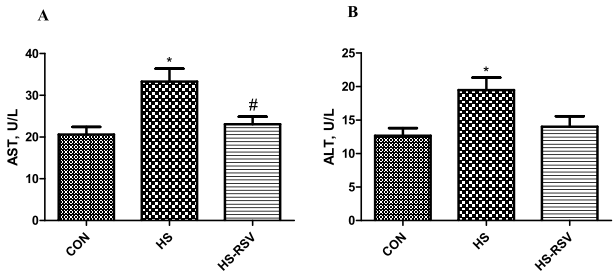

Fig. 2. Resveratrol alleviates the heat-stress-induced hepatic injury in rats evidenced by the decreased serum (A) aspartate aminotransferase (AST) and (B) alanine aminotransferase (ALT) levels. CON, rats were orally fed with 0.5% carboxymethylcellulose sodium under normal condition; HS, rats were orally fed with 0.5% carboxymethylcellulose sodium and challenged with heat treatment; HS-RSV, rats were orally fed with 100 mg resveratrol/kg body weight/day and challenged with heat treatment. The column and its bar represented the means value and standard error, n = 8, respectively. Significant difference is depicted as $^{*}p < 0.05$ when compared with the CON group, $^{\#}p < 0.05$ when compared with the HS group.

3.3. Histological analysis

In Fig. 3, histological analysis of the liver showed that the CON group had normal cell morphology. However, in the HS group, vacuolation of hepatocyte and the disorganization of parenchyma were obviously displayed when compared with the CON group. As expected, the HS-induced the alteration of hepatic histology in the HS-RSV group was partially alleviated by RSV treatment.

3.4. Hepatic redox status

Compared with the CON group, rats exposed to heat exposure had higher hepatic MDA content and GPX activity in the HS group ($p < 0.05$, Fig. 4A and B). Oral administration of RSV to heat-stressed rats led to the reduced liver MDA concentration in the HS-RSV group when compared with the HS group ($p < 0.05$). Hepatic T-SOD activity and T-AOC level were unchanged among 3 groups ($p > 0.05$, Fig. 4C and D).

3.5. Hepatic gene expression related to oxidative stress

In the liver, heat exposure resulted in the increased SOD1 and GPX1 mRNA expression concomitant with a decreased Nrf2 gene expression in the HS group when compared with the CON group ($p < 0.05$, Fig. 5). Administration of RSV decreased ($p < 0.05$) the mRNA expression of SOD1, GPX1 and Keap1 in the liver of the HS-RSV group when compared with the HS group.

3.6. Hepatic TNF-α concentration

The hepatic TNF-α concentration was higher in the HS group when compared with the CON group ($p < 0.05$, Fig. 6). However, RSV administration decreased ($p < 0.05$) the hepatic TNF-α content in the HS-RSV group when compared with the HS group.

3.7. Hepatic gene expression related to inflammation

As exhibited in Fig. 7, compared with the CON group, heat treatment increased the hepatic mRNA abundant of IFN-γ ($p = 0.056$), TNF-α ($p = 0.052$), IL10 ($p < 0.05$) and TLR4 ($p < 0.05$) in the HS group. However, rats in the HS-RSV group had lower hepatic IFN-γ ($p < 0.05$), TNF-α ($p < 0.05$), IL10 ($p < 0.05$), Ccl2 ($p = 0.062$) and TLR4 ($p < 0.05$) mRNA abundant when compared with the HS group.

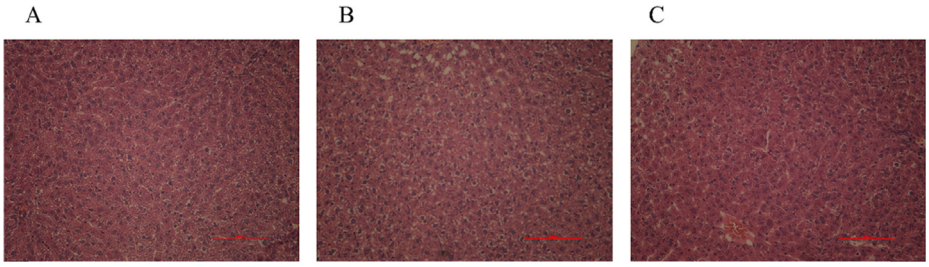

Fig. 3. Effects of resveratrol on the heat-stress-induced histological changes in liver of rats. Representative photomicrographs of livers from the different groups, stained with hematoxylin and eosin. Original magnification 200 ×, Scale bars = 100 μm. (A) CON, rats were orally fed with 0.5% carboxymethylcellulose sodium under normal condition; (B) HS, rats were orally fed with 0.5% carboxymethylcellulose sodium and challenged with heat treatment; (C) HS-RSV, rats were orally fed with 100 mg resveratrol/kg body weight/day and challenged with heat treatment.

The hepatic IL6 mRNA expression was not altered among experimental groups ($p > 0.05$).

4. Discussion

In the present study, administration of RSV to heat-stressed rats exerted a preventive and beneficial role in hepatic damage by inhibiting MDA and TNF-α concentrations, and down-regulating the expression of TLR4 and cytokines at the transcriptional level. Interestingly, although the decreased HS-induced OS in the liver was observed in the HS-RSV group, RSV treatment did not enhance the antioxidant enzymes activities and gene expression. But we could conclude that the hepatoprotective effect of RSV on heat-stressed rats is due to the decreased OS and inflammation in this study.

The expression of HSPs induced by HS is closely associated with adapted thermotolerance against a sudden heat shock (Horowitz, 2002). It is well known that the expression of HSPs is involved in a cellular defense mechanism that enables cells to deal with the stressful conditions including hyperthermia that induces OS and inflammation (Yun et al., 2012). In the HSPs family, HSP70 is the most conservative and common family, and is widely expressed in tissues (Hao et al., 2012; Song et al., 2017). Moreover, the overexpression of HSP70 has been widely regarded as a maker of HS (Yu et al., 2008). In agreement with previous studies (Hall et al., 2000; Kim et al., 2012, 2015; Yun et al., 2012), we also found that the hepatic HSP70 mRNA expression in rats was increased during HS exposure. Interestingly, administration of RSV to heat-stressed rats reduced the mRNA expression of HSP70 in the liver, which is identical to the findings in quail (Sahin et al., 2012). The results indicated that RSV can reduce the heat stress response. Previous studies have demonstrated that the maintenance of redox and immune

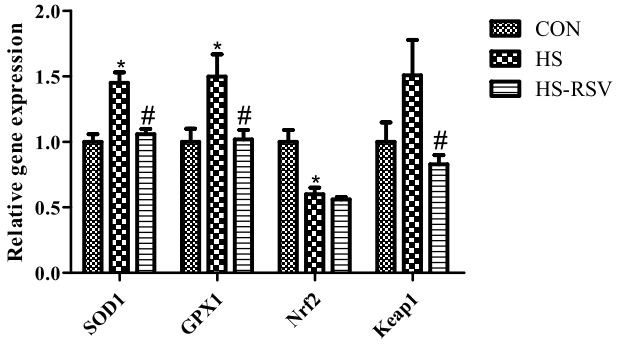

Fig. 5. Resveratrol impacts the hepatic gene expression related to oxidative stress in heat-stressed rats. CON, rats were orally fed with 0.5% carboxymethylcellulose sodium under normal condition; HS, rats were orally fed with 0.5% carboxymethylcellulose sodium and challenged with heat treatment; HS-RSV, rats were orally fed with 100 mg resveratrol/kg body weight/day and challenged with heat treatment. SOD1, superoxide dismutase 1; GPX1, glutathione peroxidase 1; Nrf2, nuclear factor, erythroid 2-like 2; Keap1, Kelch-like ECH-associated protein 1. The column and its bar represented the means value and standard error, n = 8, respectively. Significant difference is depicted as $^{*}p < 0.05$ when compared with the CON group, $^{\#}p < 0.05$ when compared with the HS group.

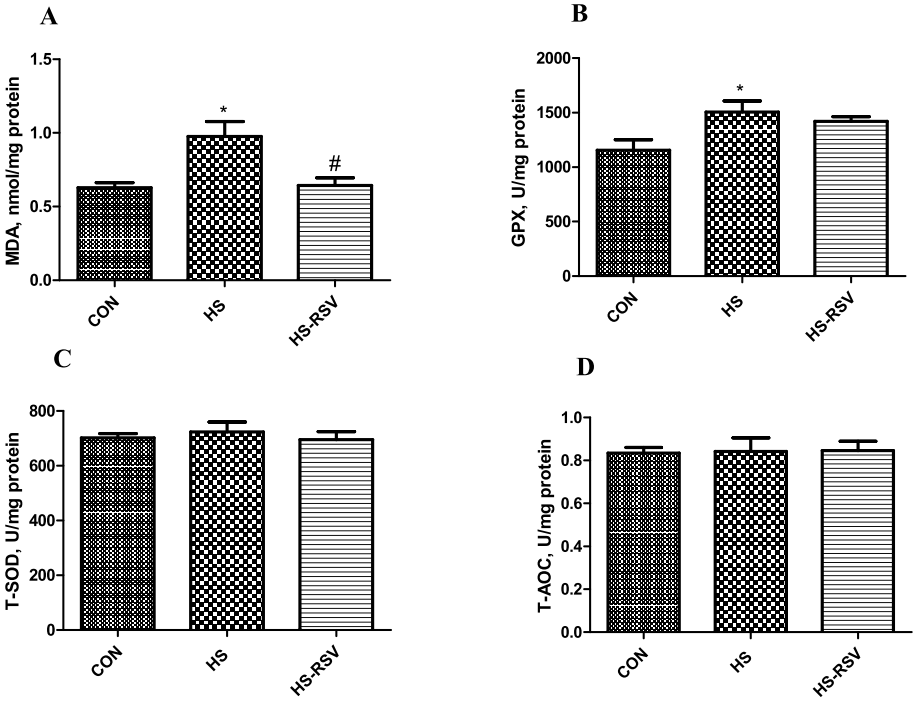

Fig. 4. Resveratrol affects the hepatic redox status in heat-stressed rats. (A) MDA, malondialdehyde; (B) GPX, glutathione peroxidase; (C) T-SOD, total superoxide dismutase; (D) T-AOC, total antioxidant capacity. CON, rats were orally fed with 0.5% carboxymethylcellulose sodium under normal condition; HS, rats were orally fed with 0.5% carboxymethylcellulose sodium and challenged with heat treatment; HS-RSV, rats were orally fed with 100 mg resveratrol/kg body weight/day and challenged with heat treatment. The column and its bar represented the means value and standard error, n = 8, respectively. Significant difference is depicted as $^{*}p < 0.05$ when compared with the CON group, $^{\#}p < 0.05$ when compared with the HS group.

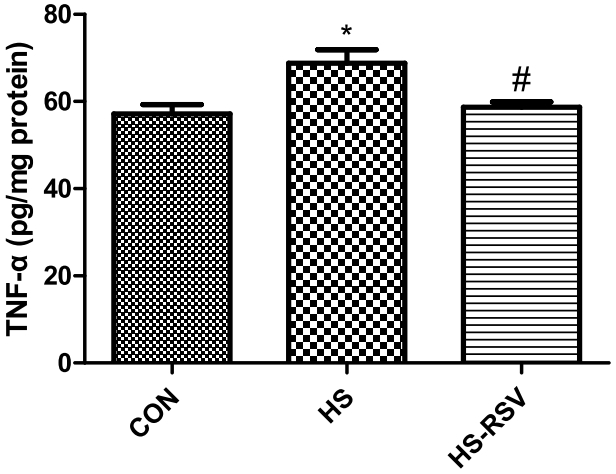

Fig. 6. Resveratrol decreases the hepatic tumor necrosis factor alpha (TNF-α) concentration in heat-stressed rats. CON, rats were orally fed with 0.5% carboxymethylcellulose sodium under normal condition; HS, rats were orally fed with 0.5% carboxymethylcellulose sodium and challenged with heat treatment; HS-RSV, rats were orally fed with 100 mg resveratrol/kg body weight/day and challenged with heat treatment. The column and its bar represented the means value and standard error, n = 8, respectively. Significant difference is depicted as $^{*}p < 0.05$ when compared with the CON group, $^{\#}p < 0.05$ when compared with the HS group.

status are beneficial for the attenuation of stress-induced HSP70 expression (Song et al., 2017, 2018). Therefore, in the present study, the regulation of HSP70 in heat-stressed rats by RSV may be attributed to the inhibition of OS and inflammation in the liver.

As we known, both AST and ALT are normally expressed in hepatocyte. However, under hepatic damage, the release of ALT and AST into the circulation results in the increased these enzymes activities in the serum (Zhang et al., 2014). Therefore, the elevated these enzymes activities in the serum are considered as a reliable and specific marker of liver injury (Dufour et al., 2000). HS induced the increased AST and ALT activities in the serum of rats, which is consistent with the findings of the previous study (Chen et al., 2013). These results suggested that HS led to hepatic damage in the present study, which also was directly supported by the histological observation. As expected, RSV administration attenuated HS-mediated liver damage evidenced by the reduced of AST and ALT activities in the serum and the improved hepatic histology. Similar study in quail (Sahin et al., 2012) showed that RSV protected against the adverse effects of HS on liver.

It has been reported that HS results in excessive ROS production owing to enhanced metabolic reaction (Yun et al., 2012). The antioxidants in cell are responsible for the elimination of free radicals. However, OS is stimulated as a result of the imbalance between ROS production and antioxidants levels. MDA, a lipid peroxidation substance, is widely used for the evaluation of OS in tissues (Cheng et al., 2017a). Similar to previous studies (Zhang et al., 2003; Kim et al., 2012), we also found that the hepatic MDA concentration was elevated by HS exposure, suggesting that HS led to hepatic OS. In addition, the increased hepatic GPX activity was observed in the HS group, which is consistent with the published literature (Yun et al., 2012; Kim et al., 2015). In this study, the increased MDA content and GPX activity in the HS group may respectively be attributed to higher free radicals levels and protective mechanism in the organism. Additionally, at the transcriptional level, HS decreased the Nrf2 expression but increased the expression of SOD1 and GPX1 in the liver of rats. Nrf2 plays an important role in preventing from the OS-induced damage by up-regulating the related antioxidants expression (Li et al., 2013). In addition to the transcriptional expression, the post-translational modification of Nrf2 is also important for the role of it in the expression of antioxidants. We speculated that there is another mechanism involved in the increased antioxidant enzyme gene expression by HS treatment, which needs to be further investigated. In this study, although the antioxidant capacity was not enhanced by RSV administration in heat-stressed rats, the hepatic MDA concentration was expectedly reduced, indicating that the liver redox status in rats subjected to HS was improved by RSV treatment. Similar results in broilers have been reported that RSV can protect against HS-induced OS in muscle (Zhang et al., 2017) and spleen (Zhang et al., 2018). In the present study, the inhibition of OS by RSV might be attributed to the beneficial role of it in inflammation, which contributes to the ROS production under HS condition (Yun et al., 2012).

In response to heat environment, a variety of cytokines (e.g., TNF-α) are known to be produced, which can contribute to hemorrhage and necrosis in multiple organs such as liver (Yun et al., 2012; Bouchama and Knochel, 2015). Moreover, evidence also supported the concept that the pro-inflammatory cytokines production is associated with the ROS and HSP70 overexpression during HS exposure (Yun et al., 2012; Song et al., 2017). Similar to the previous study of Yun et al. (2012), we also found that the increased IFN-γ, IL10 and TLR4 mRNA abundant, and the elevated protein and gene expression of TNF-α were noted in the liver of heat-stressed rats, indicating that the inflammatory cytokines induction is closely related to HS. TLR4, a stress-related biosensor in initial injury response (Mollen et al., 2006), plays a key role in stimulating pro-inflammatory cytokines production. Therefore, in the present study, the role of HS in hepatic inflammation might be due to

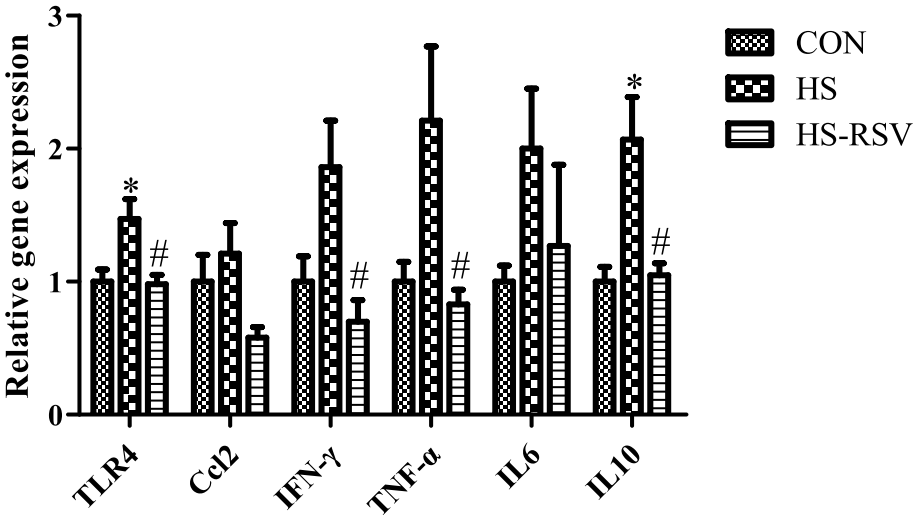

Fig. 7. Resveratrol regulates the hepatic gene expression related to inflammation in heat-stressed rats. CON, rats were orally fed with 0.5% carboxymethylcellulose sodium under normal condition; HS, rats were orally fed with 0.5% carboxymethylcellulose sodium and challenged with heat treatment; HS-RSV, rats were orally fed with 100 mg resveratrol/kg body weight/day and challenged with heat treatment. TLR4, toll-like receptor 4; Ccl2, C-C motif chemokine ligand 2; IFN-γ, interferon γ; TNF-α, tumor necrosis factor alpha; IL6, interleukin 6; IL10, interleukin 10. The column and its bar represented the means value and standard error, n = 8, respectively. Significant difference is depicted as $^{*}p < 0.05$ when compared with the CON group, $^{\#}p < 0.05$ when compared with the HS group.

the direct up-regulation of TLR4 mRNA. Expectedly, RSV treatment reduced the hepatic IFN-γ, IL10, Ccl2 and TLR4 mRNA expression, and TNF-α gene and protein levels in heat-stressed rats. In our previous study (Cheng et al., 2018), we also reported in high-fat-diet-induced obese mice that RSV administration relieved renal inflammation through inhibiting TLR4 mRNA expression. Similar results were observed in osteoarthritis of mice induced by high-fat diet (Jiang et al., 2017). Evidence also showed that the deletion of TLR4 in hepatocytes abrogated the local and systemic inflammation in mice induced by the chronic alcohol (Jia et al., 2018). Thus, RSV decreased the HS-induced hepatic inflammation may also partly be due to the suppression of TLR4 expression. This may also explain the decreased OS in the liver of the HS-RSV group.

In conclusion, the present study showed that RSV can alleviate the HS-induced hepatic damage in rats by suppressing OS and inflammation. These results suggested that RSV could be a potential therapeutic agent for the prevention and/or treatment of HS-related liver disease. Of course, the underlying mechanism of RSV in relieving the hepatic damage induced by HS in a rat model is needed to be investigated in the future.

Acknowledgement

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